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(51) International Patent Classification <sup>7</sup> : <b>C12Q 1/68, C12N 15/00, 15/09, 15/63, 15/86</b>		<b>A1</b>	(11) International Publication Number: <b>WO 00/55375</b> (43) International Publication Date: 21 September 2000 (21.09.00)
(21) International Application Number: <b>PCT/US00/07285</b> (22) International Filing Date: 17 March 2000 (17.03.00)  (30) Priority Data: 60/124,916 17 March 1999 (17.03.99) US 60/124,808 17 March 1999 (17.03.99) US 60/149,639 17 August 1999 (17.08.99) US 60/157,247 1 October 1999 (01.10.99) US 60/167,824 29 November 1999 (29.11.99) US 60/182,711 15 February 2000 (15.02.00) US  (71) Applicant: <b>ALPHAGENE, INC. [US/US]; 260 West Cummings Park, Woburn, MA 01801 (US).</b> (72) Inventors: <b>VALENZUELA, Dario; 1081 Hill Road, Boxborough, MA 01719-1010 (US). YUAN, Olive; 292 Mystic Street, Arlington, MA 02174 (US). HOFFMAN, Heidi; 90 Houghton Mill Road, Lunenburg, MA 01462 (US). HALL, Jeff; 4 Alderwood Drive, Stratham, NH 03885 (US). RAPIEJKO, Peter; 63 Old Grafton Road, Upton, MA 01568 (US).</b> (74) Agent: <b>SPRUNGER, Suzanne, A.; American Home Products Corporation, Patent &amp; Trademark Dept. - 2B, One Campus Drive, Parsippany, NJ 07054 (US).</b>		(81) Designated States: <b>AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</b>  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: <b>SECRETED PROTEINS AND POLYNUCLEOTIDES ENCODING THEM</b>			
(57) Abstract  Novel polynucleotides and the proteins encoded thereby are disclosed.			

bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLabs (Beverly, MA), Pharmacia (Piscataway, NJ) and Invitrogen Corporation (Carlsbad, CA), respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope.

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